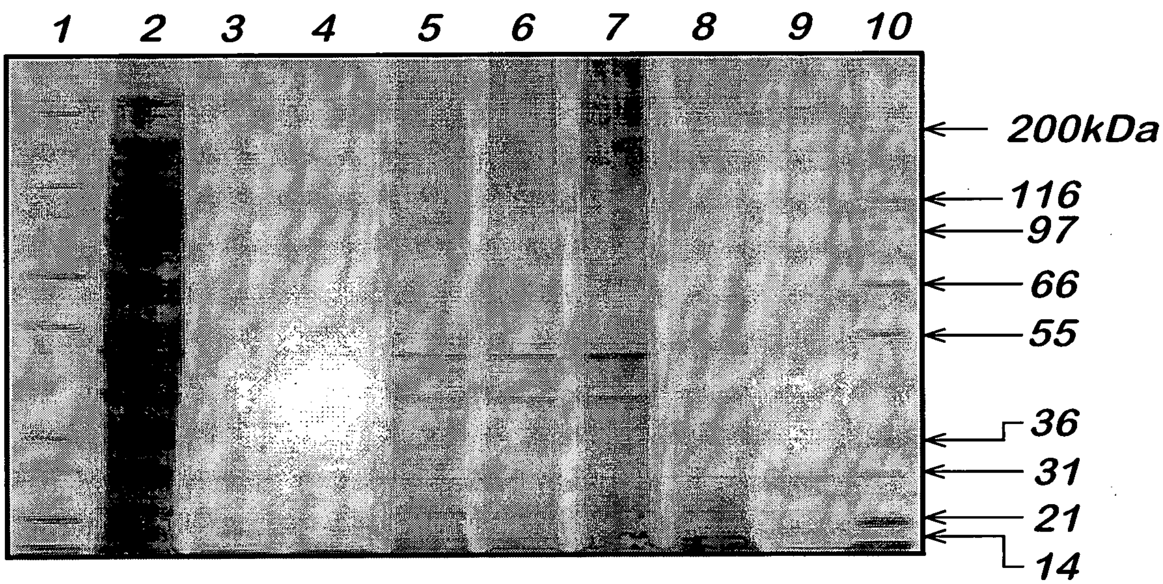
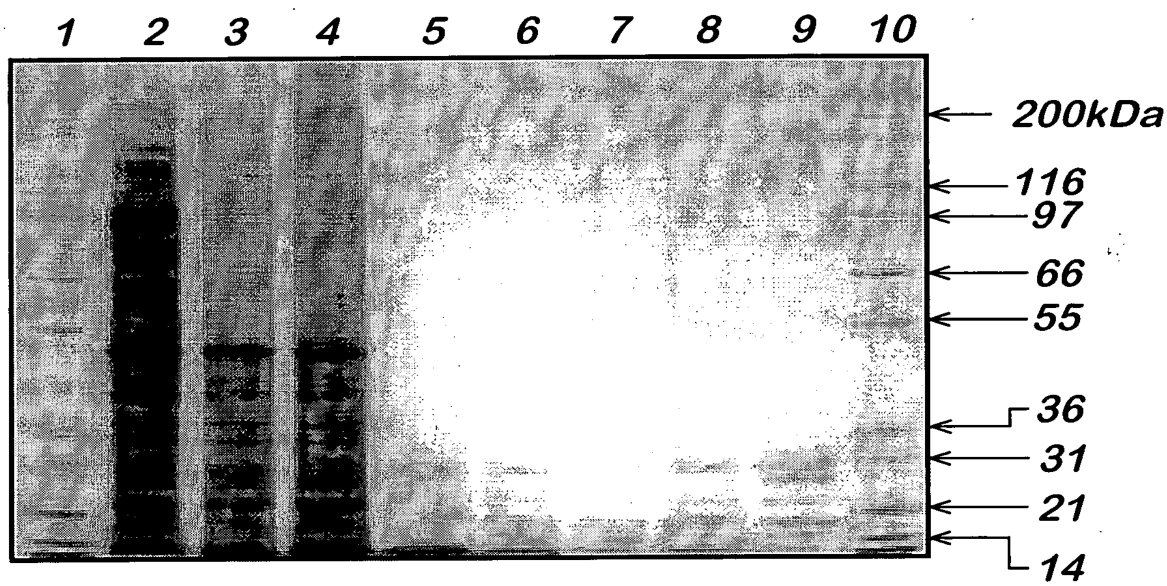
Appl. No. 10/614,370; Filed: July 8, 2003
Dkt No. 1581.0990001/RWE/VSR; Group Unit: To Be Assigned
Inventors: RAVEN, et al Tel: 202-371-2600
Title: Degradation and Detection of TSE Infectivity

Fig. 1



Lanes 1 & 10, marker proteins
Lane 2, untreated mbh
Lane 3, 50°C
Lane 4, 60°C
Lane 5, 70°C
Lane 6, 80°C
Lane 7, 90°C
Lane 8, 100°C
Lane 9, Protease M

Fig. 2



Lanes 1 & 10, marker proteins
Lane 2, untreated mbh
Lane 3, pH2
Lane 4, pH4
Lane 5, pH6
Lane 6, pH8
Lane 7, pH10
Lane 8, pH12
Lane 9, Protease M

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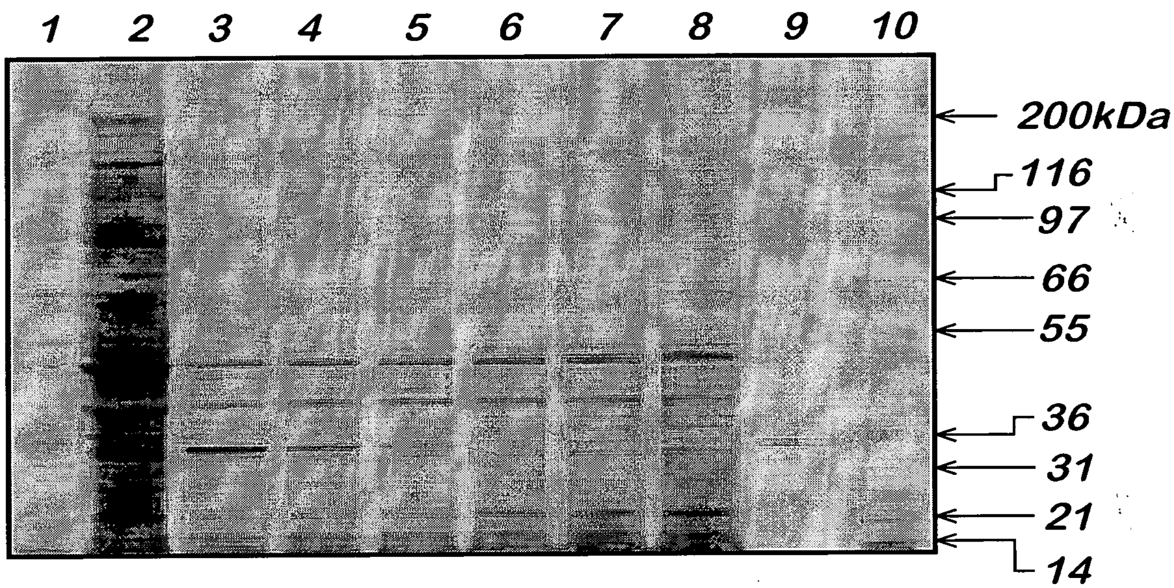
Fig. 3



Lanes 1 & 10, marker proteins
Lane 2, untreated mbh
Lanes 3 - 8, Rokko digest (20mg.ml^{-1} - 0.1 mg.ml^{-1})
Lane 9, Rokko (1mg.ml^{-1})

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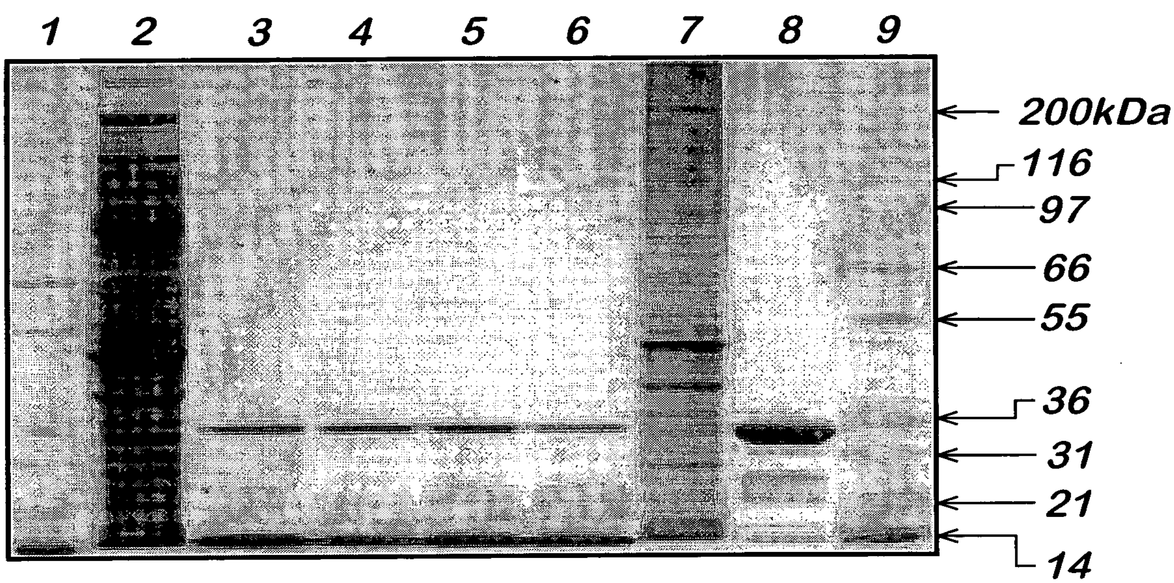
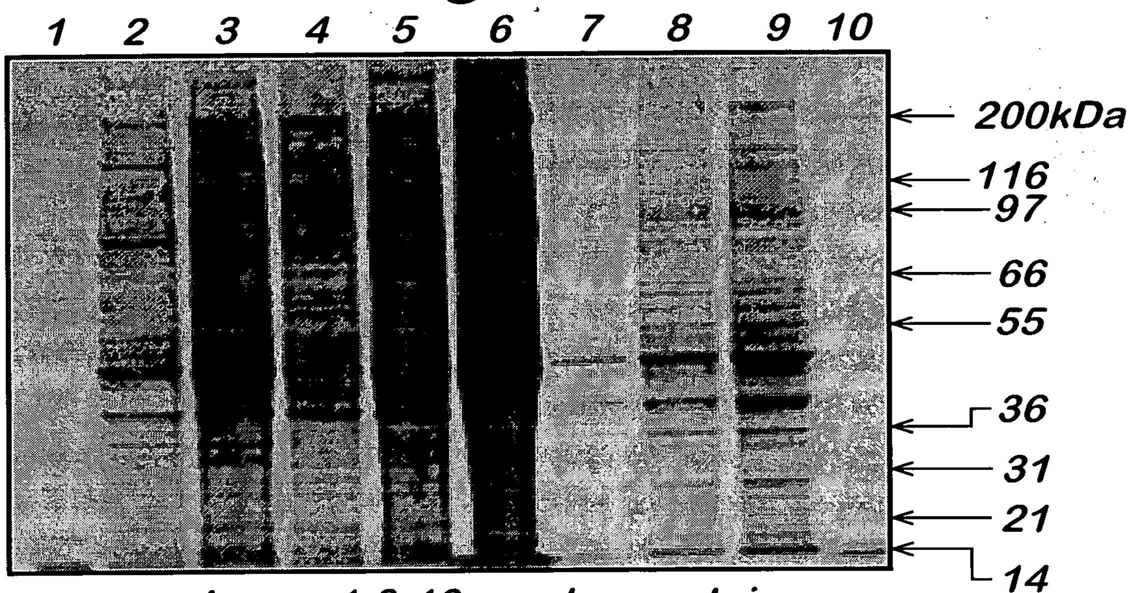
Fig. 4



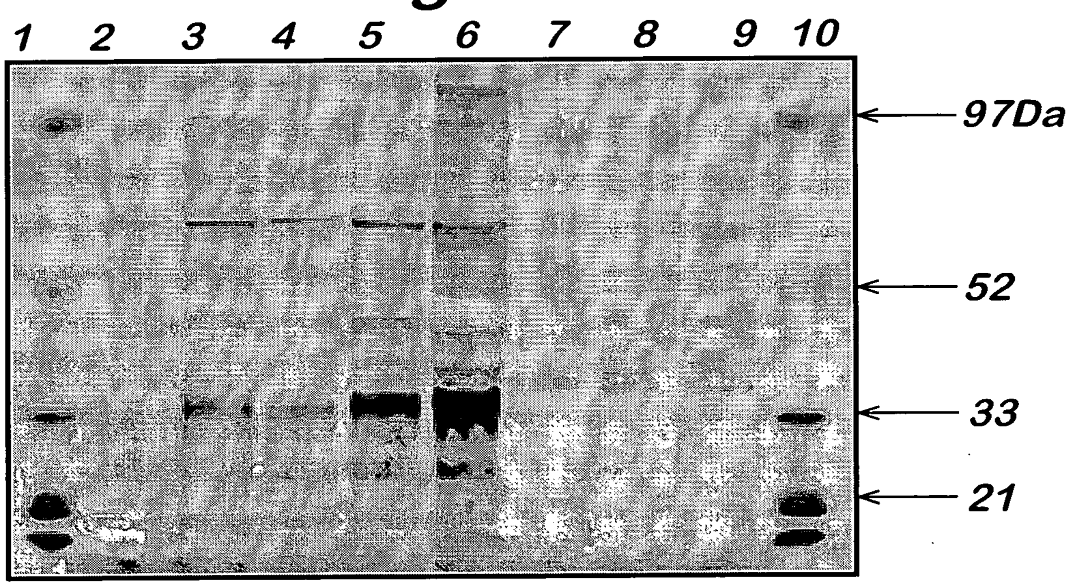
Lanes 1 & 9, marker proteins
Lane 2, untreated mbh
Lane 3, 2% SDS
Lane 4, 1% SDS
Lane 5, 0.5% SDS
Lane 6, 0.25% SDS
Lane 7, mbh + 2% SDS
Lane 8, Rokko (20mg.ml^{-1})

Fig. 5



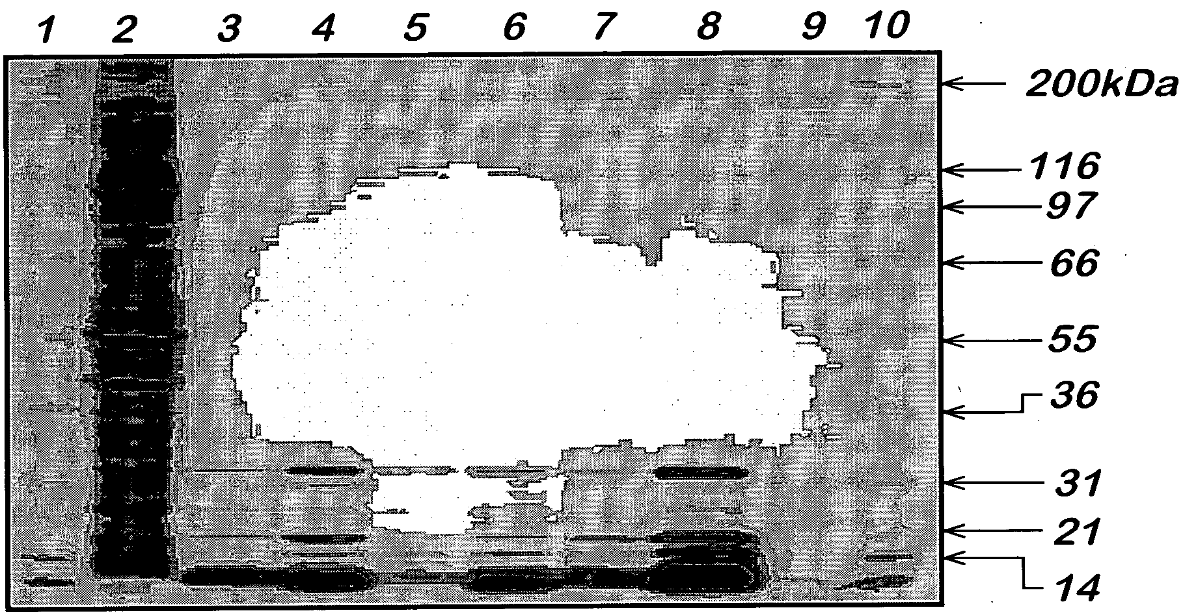
Lanes 1 & 10, marker proteins
Lanes 2 & 3, mbh
Lanes 4 - 6, mbh pellet
Lanes 7 - 9, mbh supernatant

Fig. 6



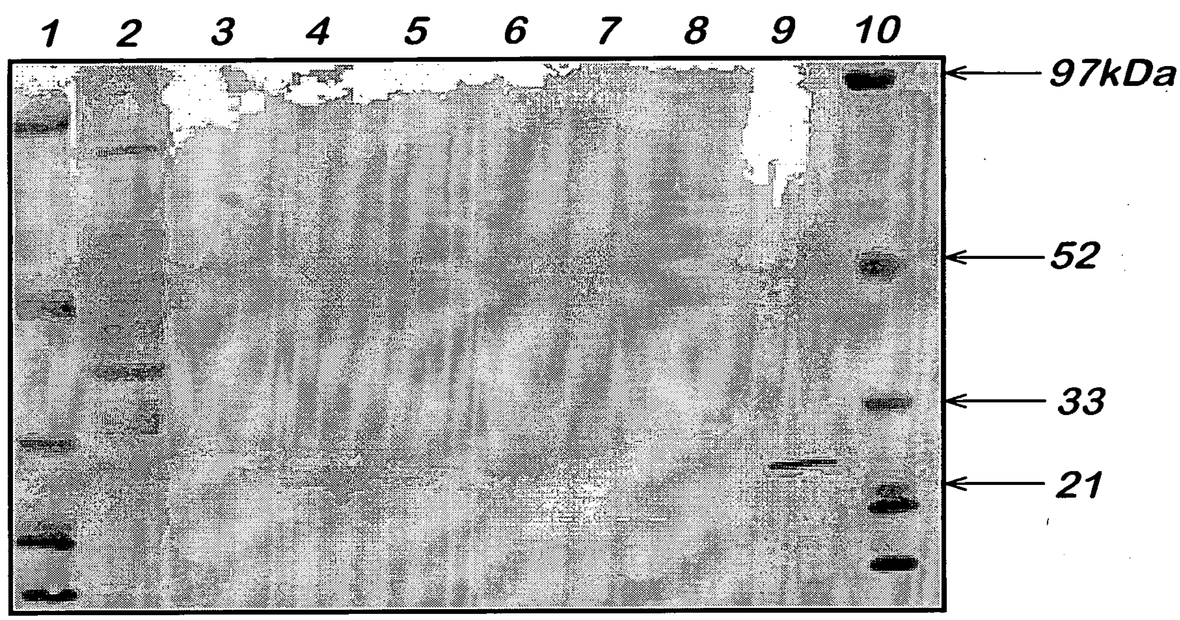
Lanes 1 & 10, marker proteins
Lanes 2 & 3, mbh
Lanes 4 - 6, mbh pellet
Lanes 7 - 9, mbh supernatant

Fig. 7



Lanes 1 & 10, marker proteins
Lane 2, untreated mbh
Lane 3, Protease G digest
Lane 4, Protease G
Lane 5, Protease R digest
Lane 6, Protease R
Lane 7, Protease C digest
Lane 8, Protease C
Lane 9, rec. mouse PrP

Fig. 8



Lanes 1 & 10, marker proteins
Lane 2, untreated mbh
Lane 3, Protease G digest
Lane 4, Protease G
Lane 5, Protease R digest
Lane 6, Protease R
Lane 7, Protease C digest
Lane 8, Protease C
Lane 9, rec. mouse PrP

Fig. 9

1 2 3 4

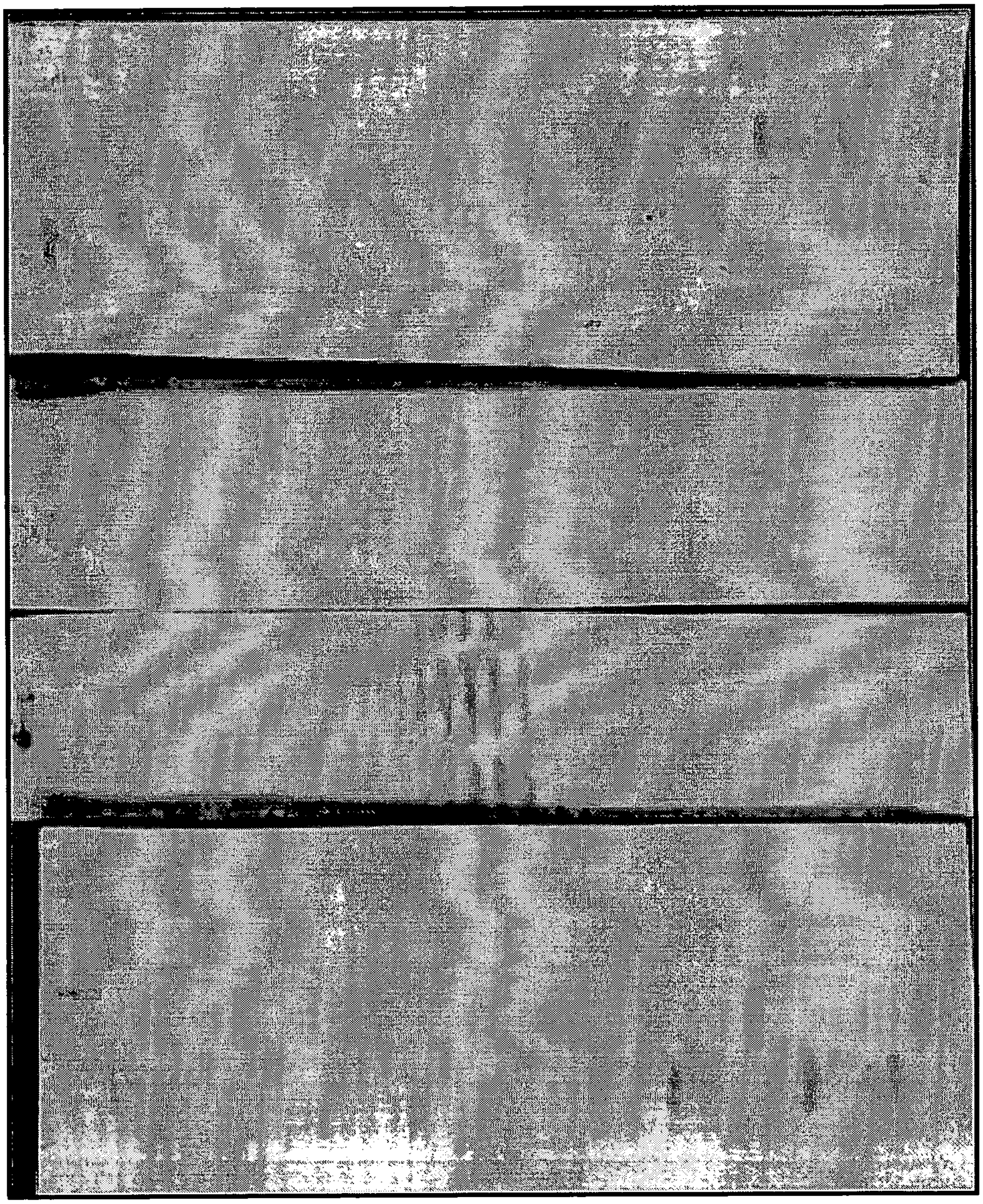


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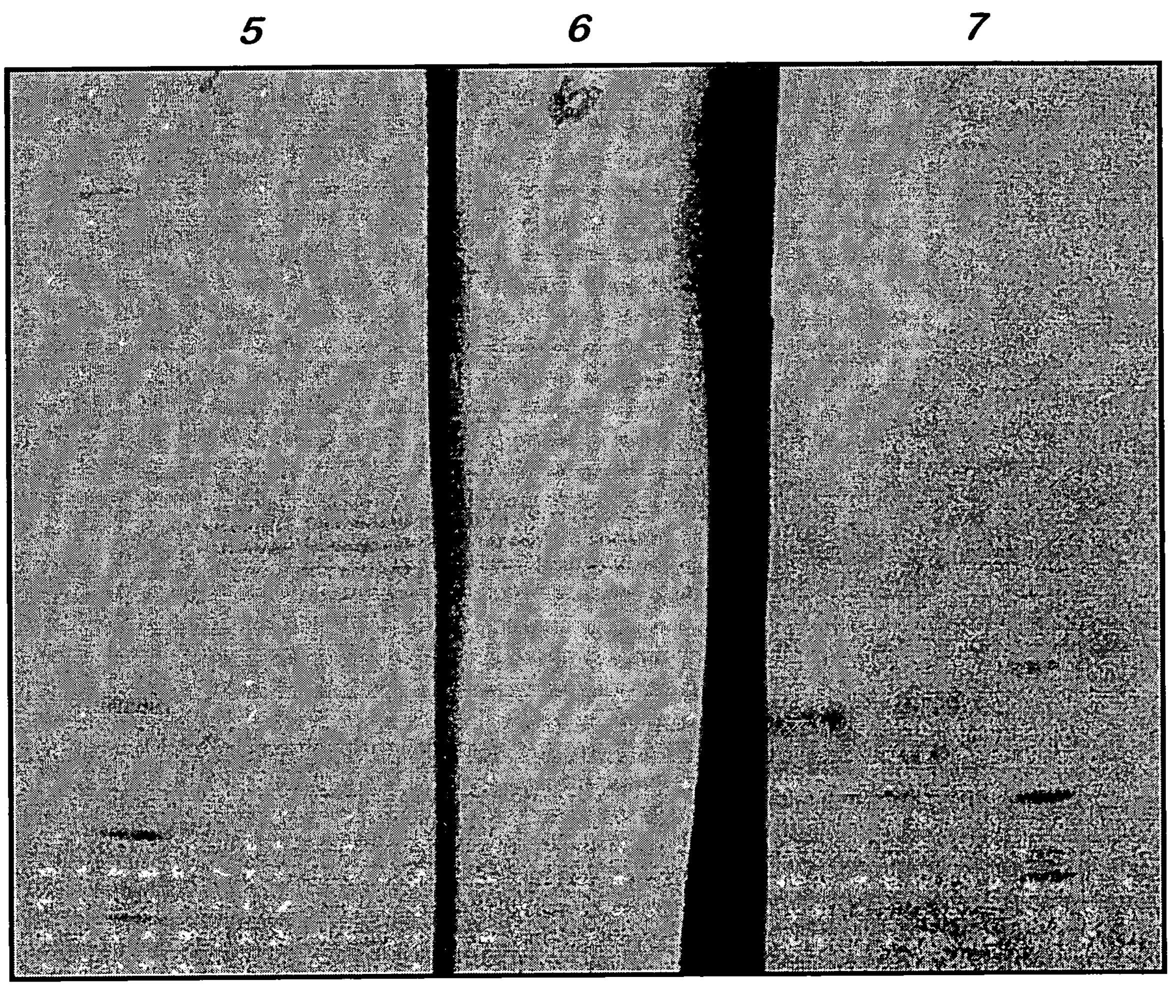
Fig. 10



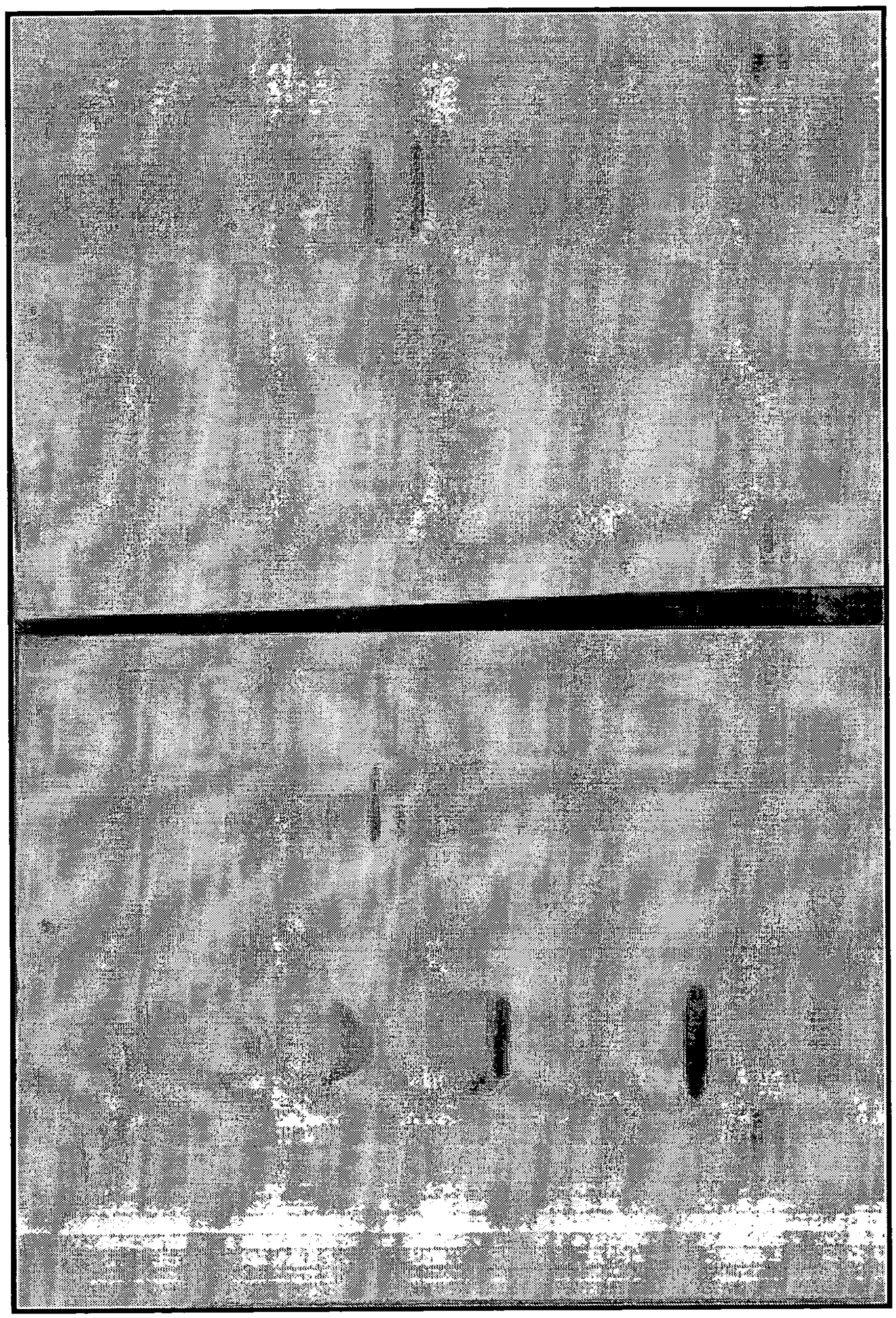


Fig. 11

Fig. 12

7

3

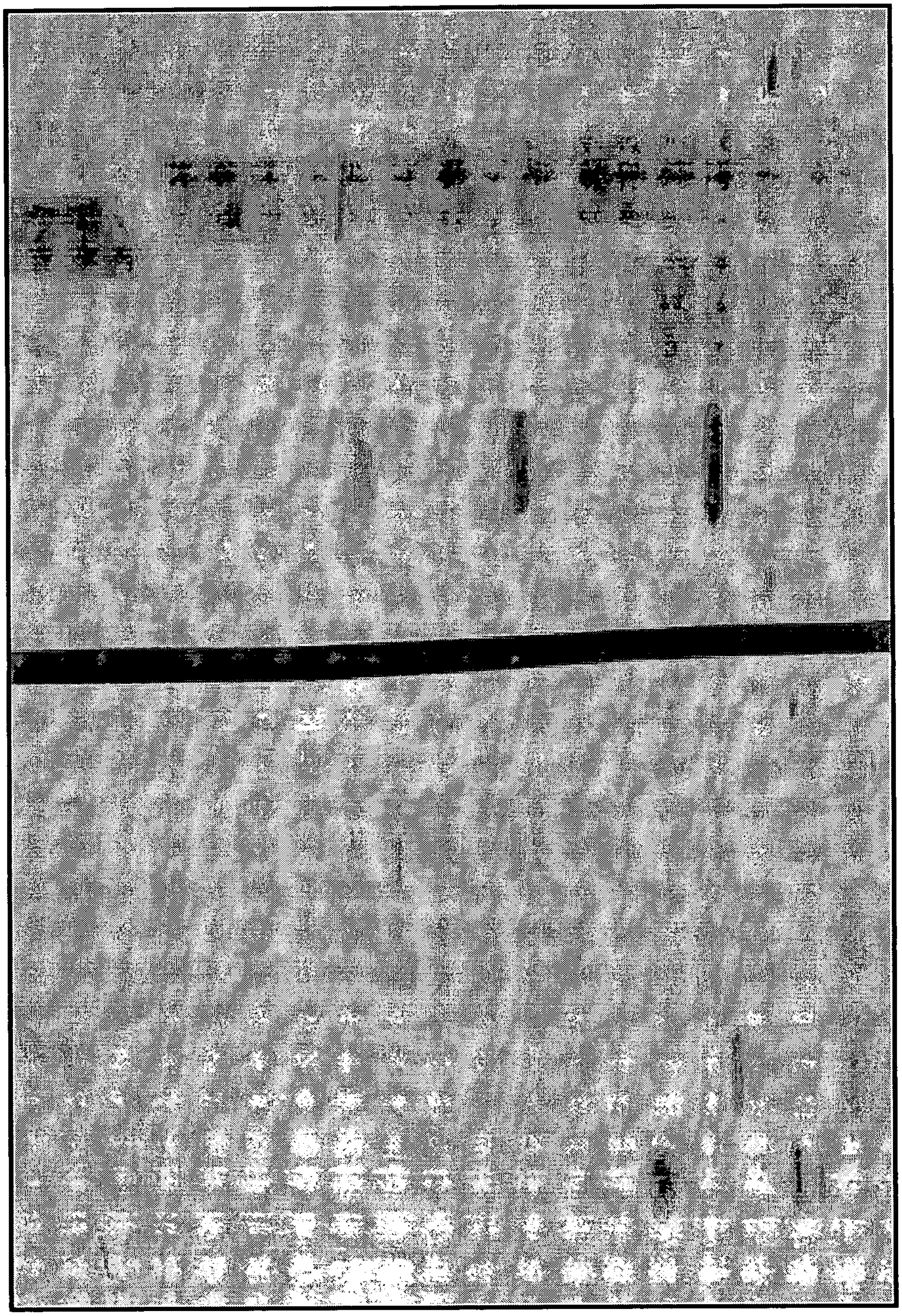


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Fig. 13.A

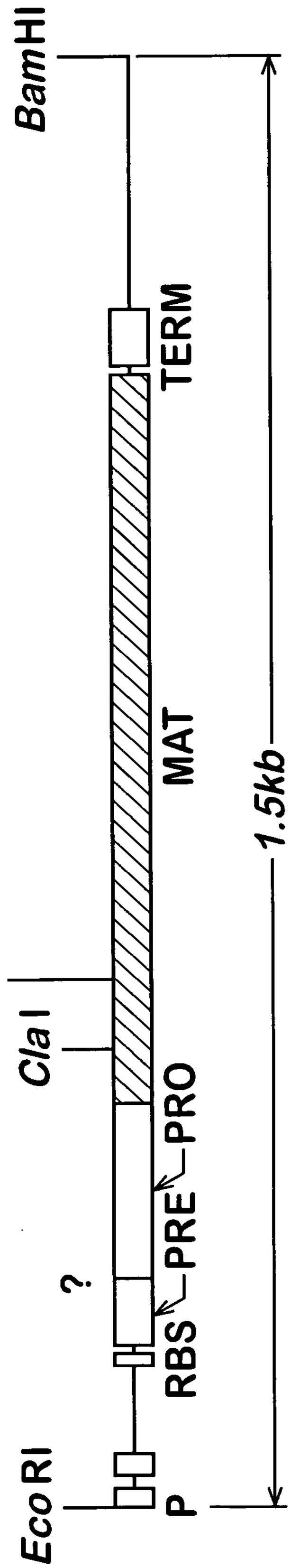

Fig. 13.B1

5 P 3 4 RBS -107 Met
1 GGTCTACTAAAATATTATTCCATACTATACAATTAATACACAGAATAATCTGTCTATTGGTTATTCTGCAAATGAAAAAAAGGAGAGGATAAAGA GTG

-100 PRE -90
Arg Gly Lys Lys Val Trp Ile Ser Leu Leu Phe Ala Leu Ala Leu Ile Phe Thr Met Ala Phe Gly Ser Thr Ser
99 AGA GGC AAA AAA GTA TGG ATC AGT TTG CTG TTT GCT TTA GCG TTA ATC TTT ACG ATG GCG TTC GGC AGC ACA TCC

-80 -70 PRO -60
Ser Ala Gln Ala Ala Gly Lys Ser Asn Gly Glu Lys Lys Tyr Ile Val Gly Phe Lys Gln Thr Met Ser Thr Met
174 TCT GCC CAG GCG GCA GGG AAA TCA AAC GGG GAA AAG AAA TAT ATT GTC GGG TTT AAA CAG ACA ATG AGC ACG ATG

-50 -40
Ser Ala Ala Lys Lys Lys Asp Val Ile Ser Glu Lys Gly Gly Lys Val Gln Lys Gln Phe Lys Tyr Val Asp Ala
249 AGC GCC GCT AAG AAG AAA GAT GTC ATT TCT GAA AAA GGC GGG AAA GTG CAA AAG CAA TTC AAA TAT GTA GAC GCA

-30 -20 -10
Ala Ser Ala Thr Leu Asn Glu Lys Ala Val Lys Glu Leu Lys Lys Asp Pro Ser Val Ala Tyr Val Glu Glu Asp
324 GCT TCA GCT ACA TTA AAC GAA AAA GCT GTA AAA GAA TTG AAA AAA GAC CCG AGC GTC GCT TAC GTT GAA GAA GAT

MAT
-1 1 10
His Val Ala His Ala Tyr Ala Gln Ser Val Pro Tyr Gly Val Ser Gln Ile Lys Ala Pro Ala Leu His Ser Gln
399 CAC GTA GCA CAT GCG TAC GCG CAG TCC GTG CCT TAC GGC GTA TCA CAA ATT AAA GCC CCT GCT CTG CAC TCT CAA

20 30 40
Gly Tyr Thr Gly Ser Asn Val Lys Val Ala Val Ile Asp Ser Gly Ile Asp Ser Ser His Pro Asp Leu Lys Val
474 GGC TAC ACT GGA TCA AAT GTT AAA GTA GCG GTT ATC GAC AGC GGT ATC GAT TCT TCT CAT CCT GAT TTA AAG GTA

Fig. 13.B2

						50						Pro	Asn			60	Asp								
	Ala	Gly	Gly	Ala	Ser	Met	Val	Pro	Ser	Glu	Thr	Asn	Pro	Phe	Gln	Asp	Asn	Asn	Ser	His	Gly	Thr	His	Val	Ala
549	GCA	GGC	GGA	GCC	AGC	ATG	GTT	CCT	TCT	GAA	ACA	AAT	CCT	TTC	CAA	GAC	AAC	AAC	TCT	CAC	GGA	ACT	CAC	GTT	GCC
	70										80									Ser	Ala	90			
	Gly	Thr	Val	Ala	Ala	Leu	Asn	Asn	Ser	Ile	Gly	Val	Leu	Gly	Val	Ala	Pro	Ser	Ala	Ser	Leu	Tyr	Ala	Val	Lys
o24	GGC	ACA	GTT	GCG	GCT	CTT	AAT	AAC	TCA	ATC	GGT	GTA	TTA	GGC	GTT	GCG	CCA	AGC	GCA	TCA	CTT	TAC	GCT	GTA	AAA
				Asp	Ala	100										110									
	Val	Leu	Gly	Ala	Asp	Gly	Ser	Gly	Gln	Tyr	Ser	Trp	Ile	Ile	Asn	Gly	Ile	Glu	Trp	Ala	Ile	Ala	Asn	Asn	Met
699	GTT	CTC	GGT	GCT	GAC	GGT	TCC	GGC	CAA	TAC	AGC	TGG	ATC	ATT	AAC	GGA	ATC	GAG	TGG	GCG	ATC	GCA	AAC	AAT	ATG
	120										130										140				
	Asp	Val	Ile	Asn	Met	Ser	Leu	Gly	Gly	Pro	Ser	Gly	Ser	Ala	Ala	Leu	Lys	Ala	Ala	Val	Asp	Lys	Ala	Val	Ala
774	GAC	GTT	ATT	AAC	ATG	AGC	CTC	GGC	GGA	CCT	TCT	GGT	TCT	GCT	GCT	TTA	AAA	GCG	GCA	GTT	GAT	AAA	GCC	GTT	GCA
						150								Ser	Thr	160									
	Ser	Gly	Val	Val	Val	Val	Ala	Ala	Ala	Gly	Asn	Glu	Gly	Thr	Ser	Gly	Ser	Ser	Ser	Thr	Val	Gly	Tyr	Pro	Gly
849	TCC	GGC	GTC	GTA	GTC	GTT	GCG	GCA	GCC	GGT	AAC	GAA	GGC	ACT	TCC	GGC	AGC	TCA	AGC	ACA	GTG	GGC	TAC	CCT	GGT
	170										180										190				
	Lys	Tyr	Pro	Ser	Val	Ile	Ala	Val	Gly	Ala	Val	Asp	Ser	Ser	Asn	Gln	Arg	Ala	Ser	Phe	Ser	Ser	Val	Gly	Pro
924	AAA	TAC	CCT	TCT	GTC	ATT	GCA	GTA	GGC	GCT	GTT	GAC	AGC	AGC	AAC	CAA	AGA	GCA	TCT	TTC	TCA	AGC	GTA	GGA	CCT
						200										210									
	Glu	Leu	Asp	Val	Met	Ala	Pro	Gly	Val	Ser	Ile	Gln	Ser	Thr	Leu	Pro	Gly	Asn	Lys	Tyr	Gly	Ala	Tyr	Asn	Gly
999	GAG	CTT	GAT	GTC	ATG	GCA	CCT	GGC	GTA	TCT	ATC	CAA	AGC	ACG	CTT	CCT	GGA	AAC	AAA	TAC	GGG	GCG	TAC	AAC	GGT
	220										230										240				
	Thr	Ser	Met	Ala	Ser	Pro	His	Val	Ala	Gly	Ala	Ala	Ala	Leu	Ile	Leu	Ser	Lys	His	Pro	Asn	Trp	Thr	Asn	Thr
1074	ACG	TCA	ATG	GCA	TCT	CCG	CAC	GTT	GCC	GGA	GCG	GCT	GCT	TTG	ATT	CTT	TCT	AAG	CAC	CCG	AAC	TGG	ACA	AAC	ACT

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```
                                 250  Gln                                         260
     Gln  Val  Arg  Ser  Ser  Leu  Glu  Asn  Thr  Thr  Thr  Lys  Leu  Gly  Asp  Ser  Phe  Tyr  Tyr  Gly  Lys  Gly  Leu  Ile  Asn
1149 CAA  GTC  CGC  AGC  AGT  TTA  GAA  AAC  ACC  ACT  ACA  AAA  CTT  GGT  GAT  TCT  TTC  TAC  TAT  GGA  AAA  GGG  CTG  ATC  AAC

     270                 275
     Val  Gln  Ala  Ala  Ala  Gln  OC                TERM
1224 GTA  CAG  GCG  GCA  GCT  CAG  TAA  AACATAAAAAACCGGCCTTGGCCCCGCCGGTTTTTTATTTTTCTTCCTCCGCATGTTCAATCCGCTCC

1316 ATAATCGACGGATGGCTCCCTCTGAAAATTTTAACGAGAAACGGCGGGTTGACCCGGCTCAGTCCCGTAACGGCCAAGTCCTGAAACGTCTCAATCGCCG

1416 CTTCCCGGTTTCCGGTCAGCTCAATGCCGTAACGGTCGGCGGCGTTTTCCTGATACCGGGAGACGGCATTCGTAATCGGATC
```

Fig. 13.B3

Fig. 14

CONSERVED RESIDUES IN SUBTILISINS FROM *BACILLUS AMYLOLIQUEFACIENS*

```
1                  10                  20
A Q S V P . G . . . . . A P A . H . . G

21                 30                  40
. T G S . V K V A V . D . G . . . . H P

41                 50                  60
D L . . . G G A S . V P . . . . . . Q D

61                 70                  80
. N . H G T H V A G T . A A L N N S I G

81                 90                 100
V L G V A P S A . L Y A V K V L G A . G

101               110                 120
S G . . S . L . . G . E W A . N . . . .

121               130                 140
V . N . S L G . P S . S . . . . . A . .

141               150                 160
. . . . . G V . V V A A . G N . G . . .

161               170                 180
. . . . . . Y P . . Y . . . . A V G A .

181               190                 200
D . . N . . A S F S . . G . . L D . . A

201               210                 220
P G V . . Q S T . P G . . Y . . . N G T

221               230                 240
S M A . P H V A G A A A L . . . K . . .

241               250                 260
W . . . Q . R . . L . N T . . . L G . .

261               270
. . Y G . G L . N . . A A . .
```

Fig. 15.A

Comparison of subtilisin sequences from:
B. amyloliquefaciens   B. subtilis   B. licheniformis   B. lentus

```
01               10                  20                  30
A Q S V P Y G V S Q I K A P A L H S Q G Y T G S N V K V A V I D S G I D S S H P
A Q S V P Y G I S Q I K A P A L H S Q G Y T G S N V K V A V I D S G I D S S H P
A Q T V P Y G I P L I K A D K V Q A Q G F K G A N V K V A V L D T G I Q A S H P
A Q S V P W G I S R V Q A P A A H N R G L T G S G V K V A V L D T G I S T   H P

41               50                  60                  70
D L K V A G G A S M V P S E T N P F Q D N N S H G T H V A G T V A A L N N S I G
D L N V R G G A S F V P S E T N P Y Q D G S S H G T H V A G T I A A L N N S I G
D L N V V G G A S F V A G E A Y N * T D G N G H G T H V A G T V A A L D N T T G
D L N I R G G A S F V P G E * P S T Q D G N G H G T H V A G T I A A L N N S I G

81               90                  100                 110
V L G V A P S A S L Y A V K V L G A D G S G Q Y S W I I N G I E W A I A N N M D
V L G V S P S A S L Y A V K V L D S T G S G Q Y S W I I N G I E W A I S N N M D
  L G V A P S V S L Y A V K V L N S S G S G S Y S G I V S G I E W A T T N G M D
V L G V A P S A E L Y A V K V L G A S G S G S V S S I A Q G L E W A G N N G M H

121              130                 140                 150
V I N M S L G G P S G S A A L K A A V D K A V A S G V V V V A A A G N E G T S G
V I N M S L G G P T G S T A L K T V V D K A V S S G I V V A A A A G N E G S S G
V I N M S L G G A S G S T A M K Q A V D N A Y A R G V V V V A A A G N S G N S G
V A N L S L G S P S P S A T L E Q A V N S A T S R G V L V V A A S G N S G A G S
```

```
161                 170                 180                 190
S S S T V G Y P G K Y P S V I A V G A V D S S N Q R A S F S S V G P E L D V M A
S T S T V G Y P A K Y P S T I A V G A V N S S N Q R A S F S S A G S E L D V M A
S T N T I G Y P A K Y D S V I A V G A V D S N S N R A S F S S V G A E L E V M A
* * * * I S Y P A R Y A N A M A V G A T D Q N N N R A S F S Q Y G A G L D I V A

201                 210                 220                 230
P G V S I Q S T L P G N K Y G A Y N G T S M A S P H V A G A A A L I L S K H P N
P G V S I Q S T L P G G T Y G A Y N G T S M A T P H V A G A A A L I L S K H P T
P G A G V Y S T Y P T N T Y A T L N G T S M A S P H V A G A A A L I L S K H P N
P G V N V Q S T Y P G S T Y A S L N G T S M A T P H V A G A A A L V K Q K N P S

241                 250                 260                 270
W T N T Q V R S S L E N T T T K L G D S F Y Y G K G L I N V Q A A A Q
W T N A Q V R D R L E S T A T Y L G N S F Y Y G K G L I N V Q A A A Q
L S A S Q V R N R L S S T A T Y L G S S F Y Y G K G L I N V E A A A Q
W S N V Q I R N H L K N T A T S L G S T N L Y G S G L V N A E A A T R
```

Fig. 15.B

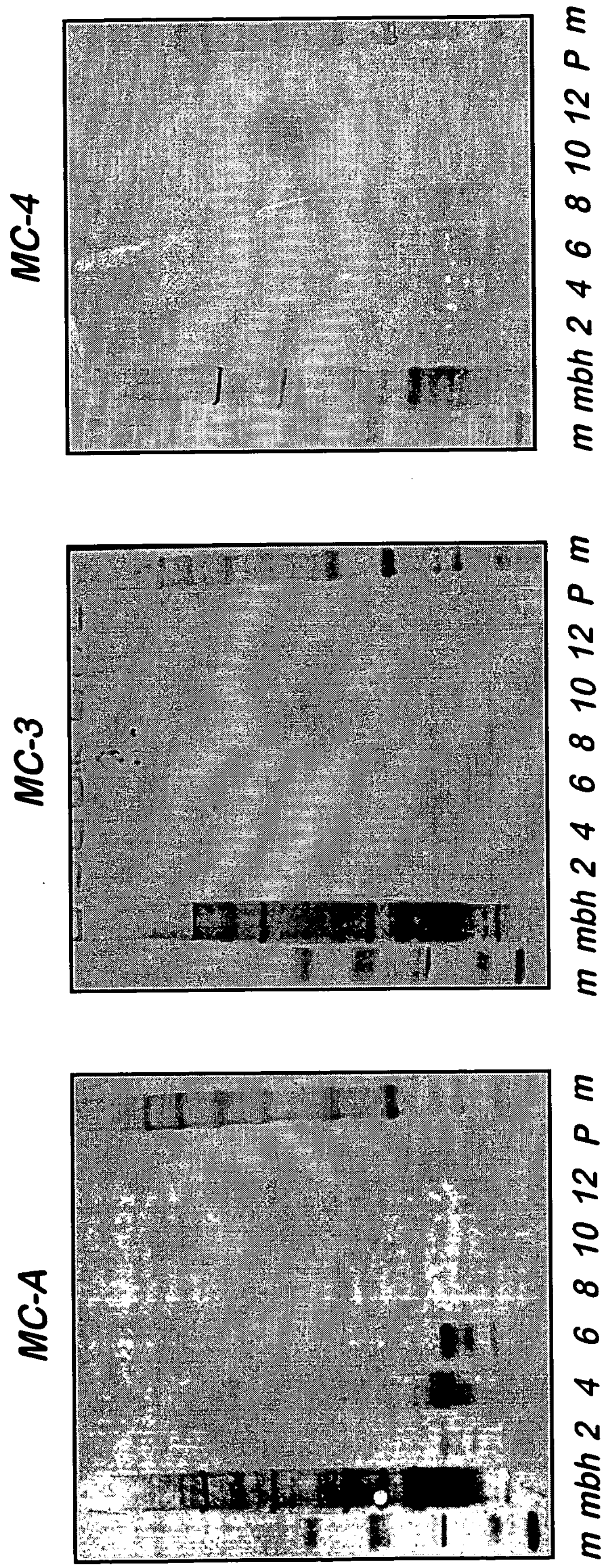

Fig. 16
Initial evaluation results

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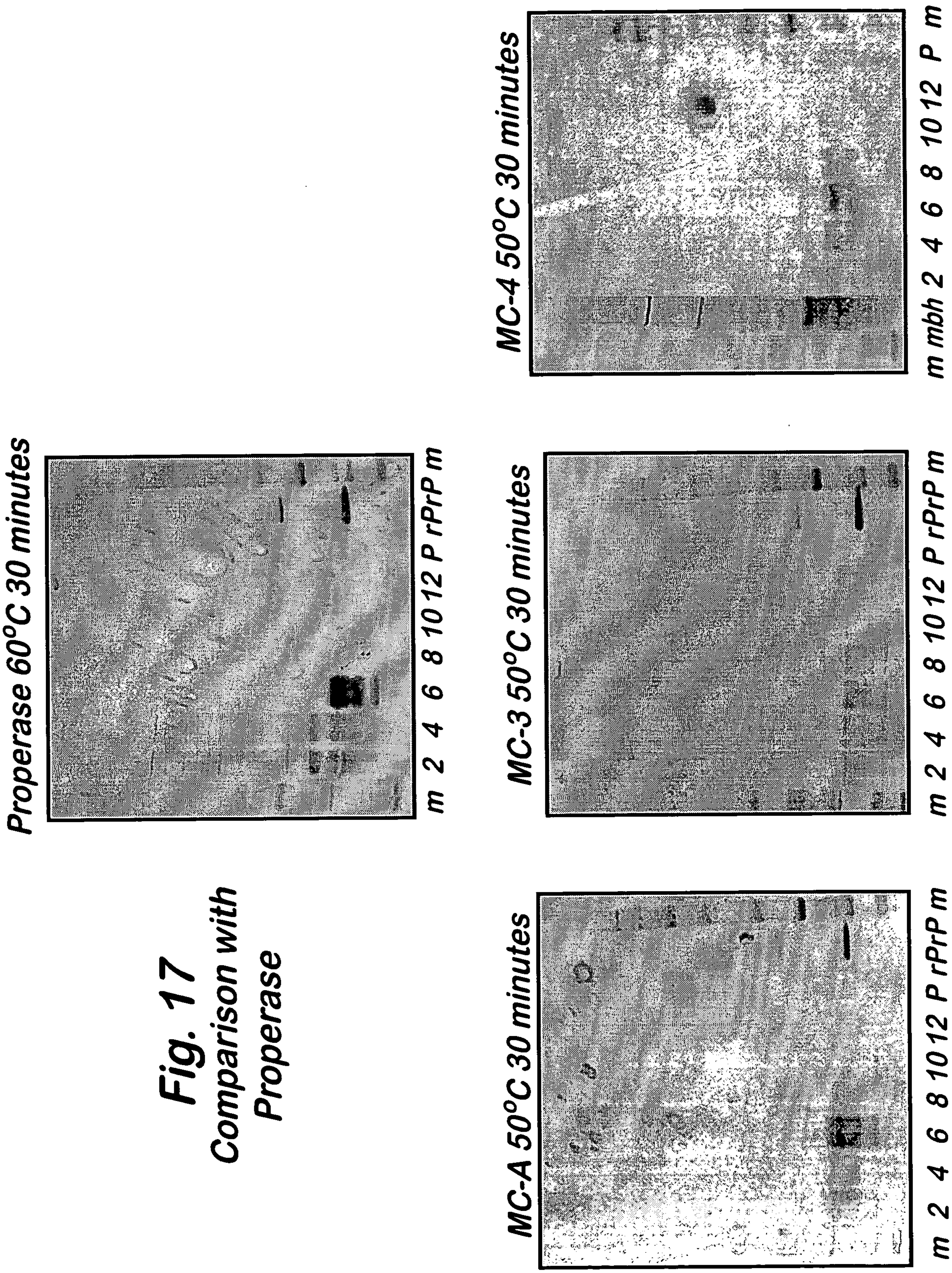

Fig. 17
Comparison with Properase

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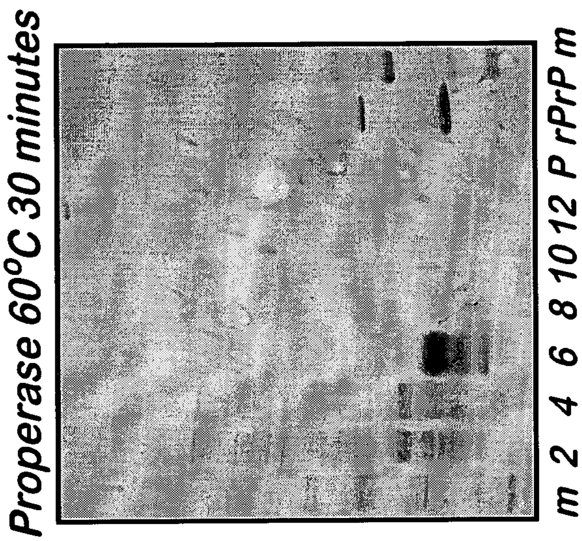
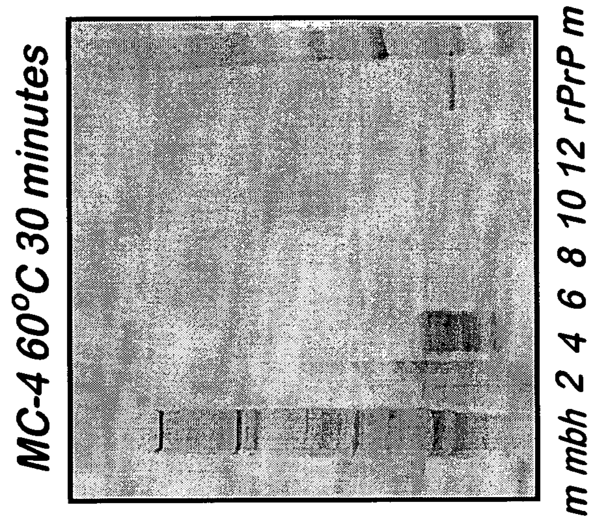
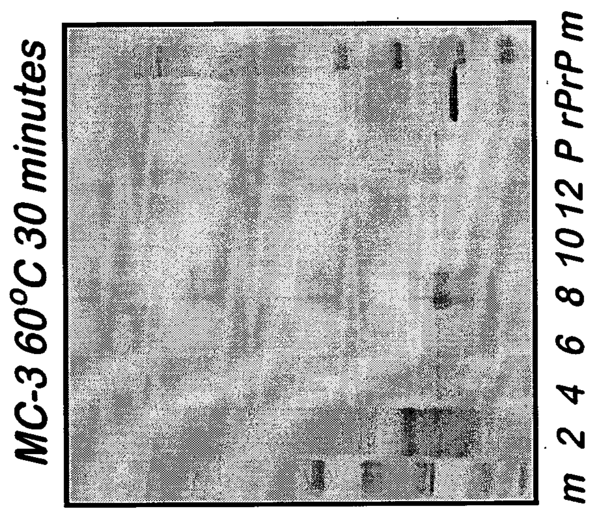
Fig. 18
Comparison with Properase







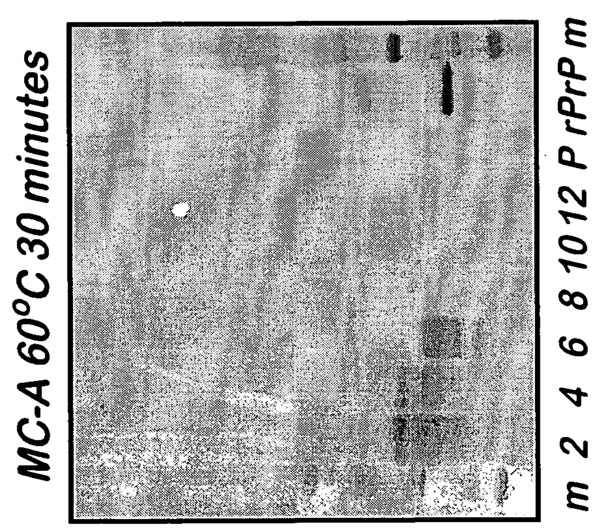


Fig. 19

Temperature profiling with MC-3

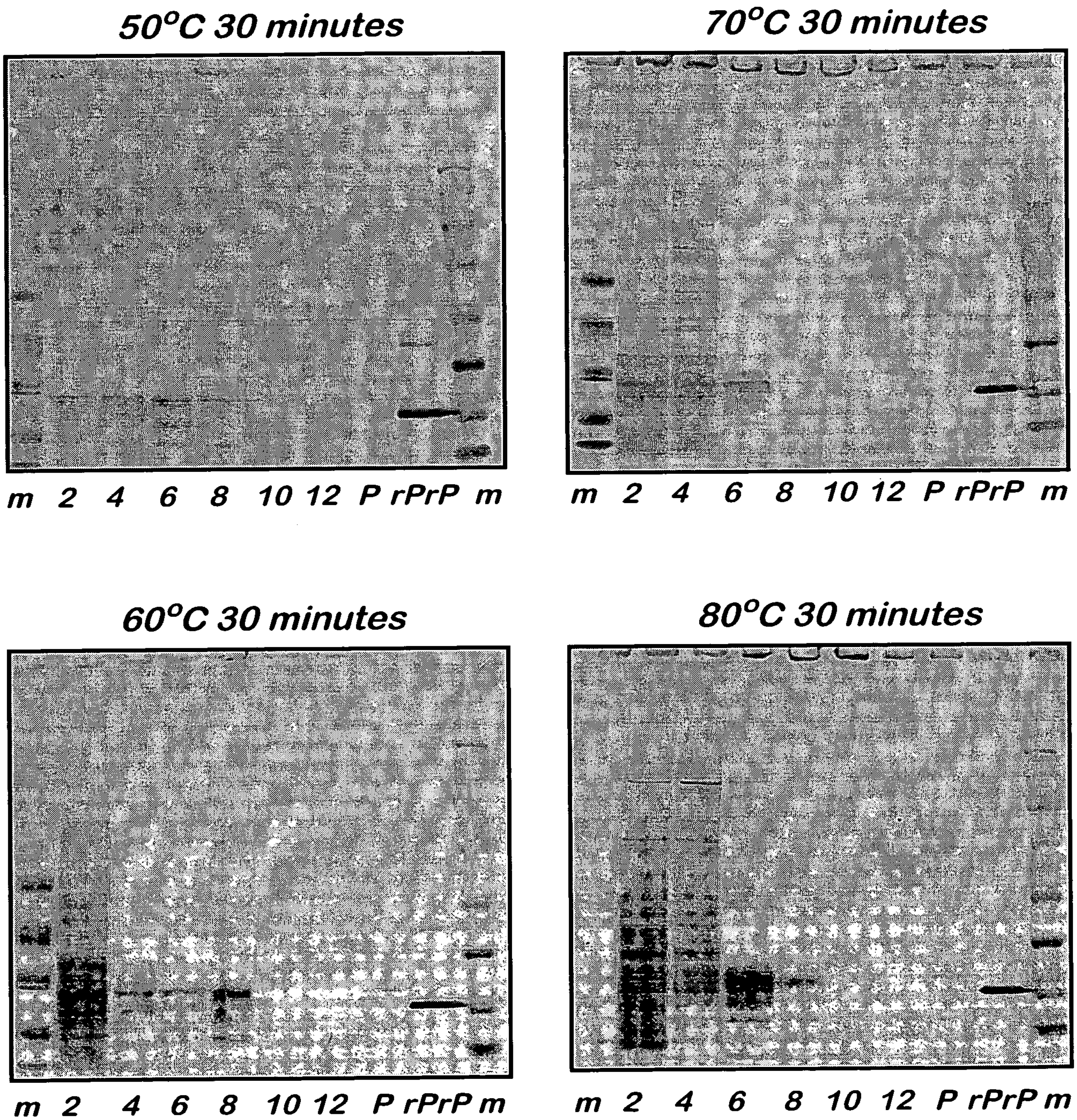


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Fig. 20

Detection with PAb2

mbh pH 2-12 digested at 50 ^{o}C 30 minutes

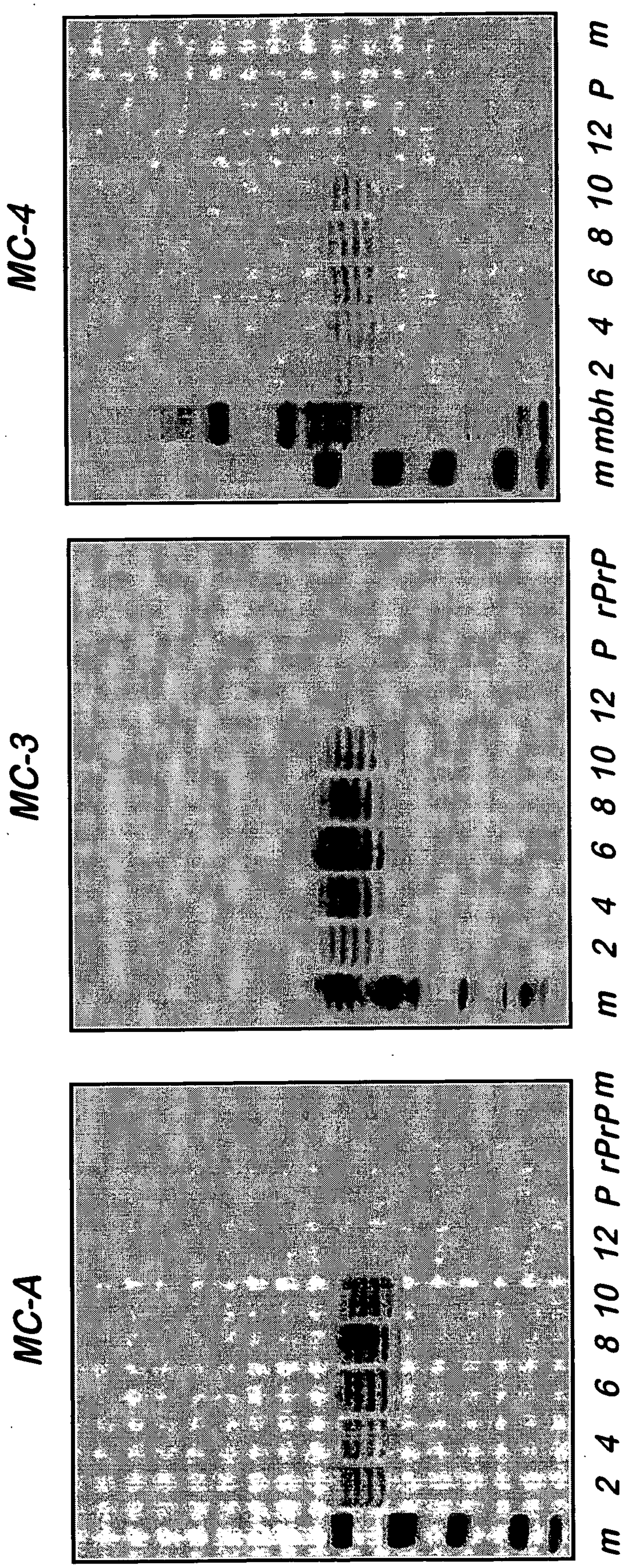

Fig. 21
MC-3 dilutions at pH10 & pH12

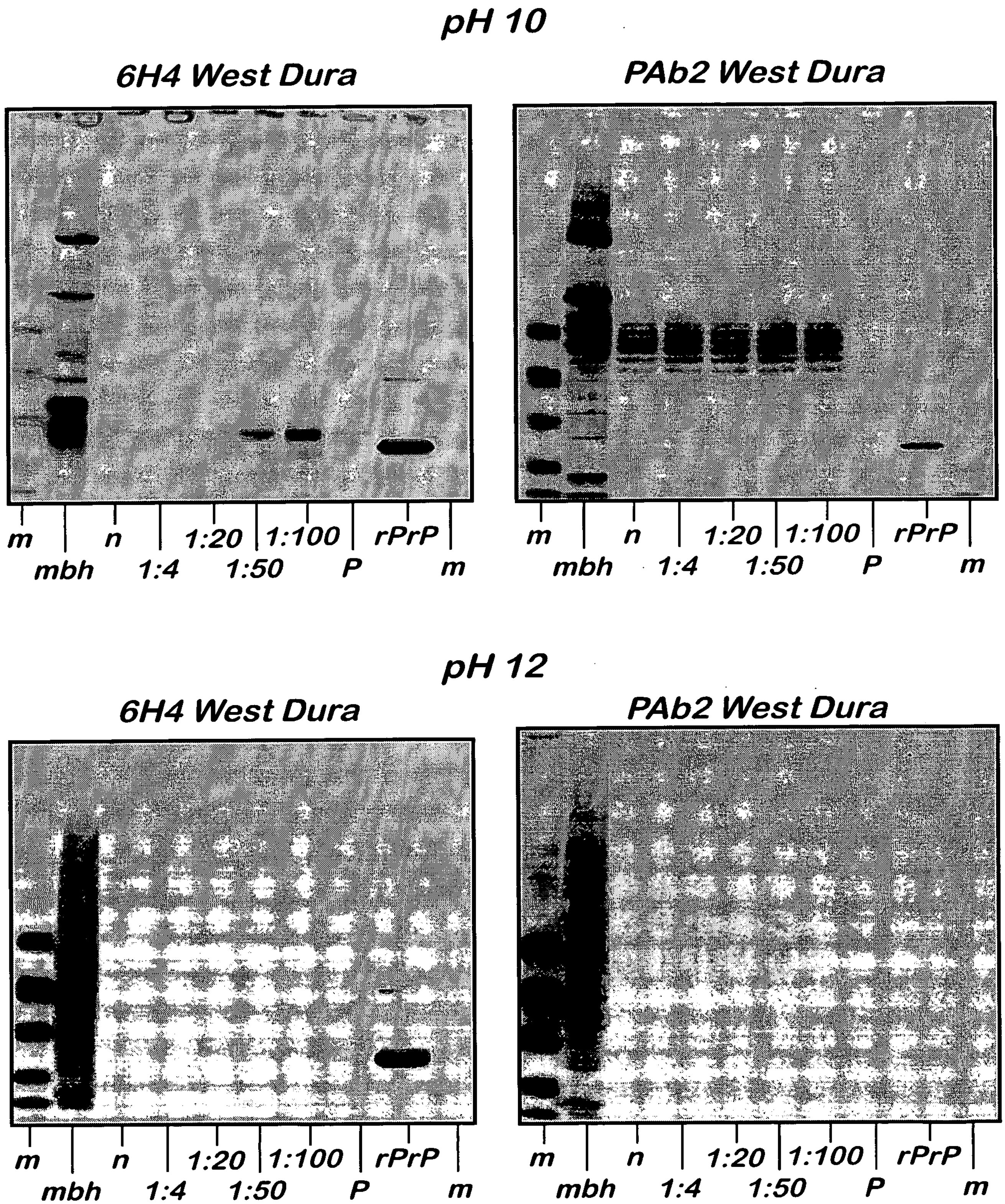

Fig. 22

Comparison with Proteinase K

Characteristic PrP^{Sc} monomer bands pH 2-10
Incomplete digestion pH12 however no clear monomers
HMW bands present pH 2-12